

Are We Spreading Antibiotic Resistance in the Environment with Recycled Wastewater? Separating Knowns from Unknowns



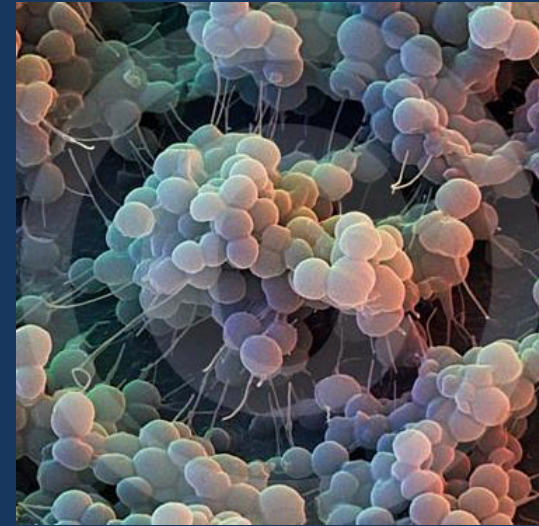
Jean E. McLain, Associate Director and Research Scientist
University of Arizona Water Resources Research Center

Presented to Idaho DEQ Water Reuse Conference
May 18, 2017

Outline

What is **antibiotic resistance**?

Antibiotic resistance in the **news**

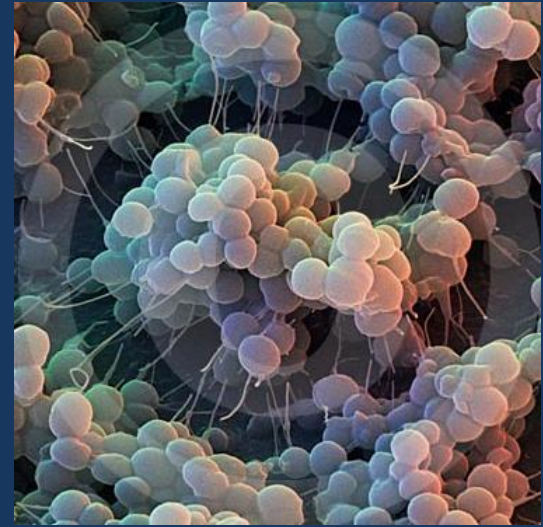


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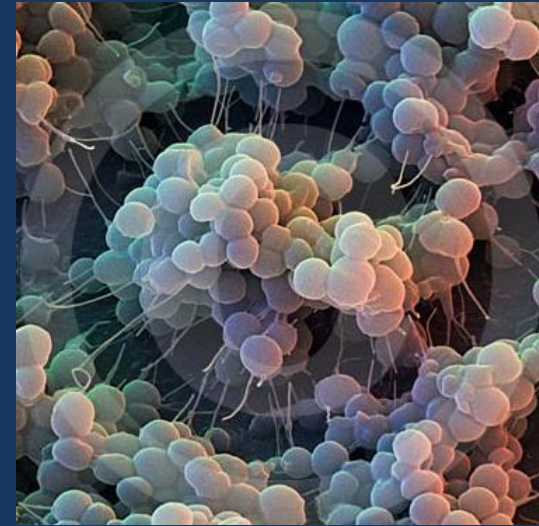
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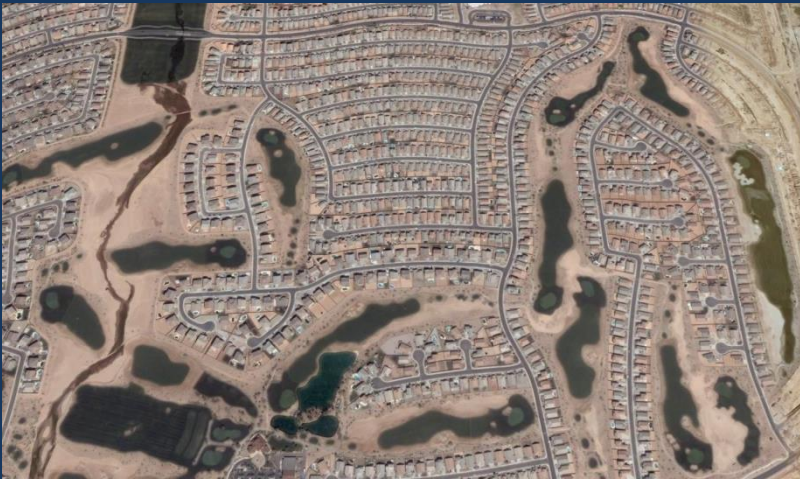
What is known and not known about antibiotic resistance and wastewater?



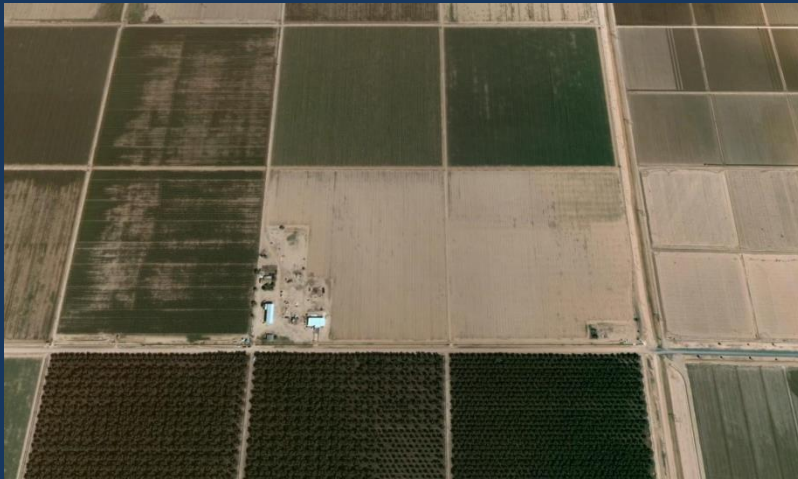
Why Water Recycling?



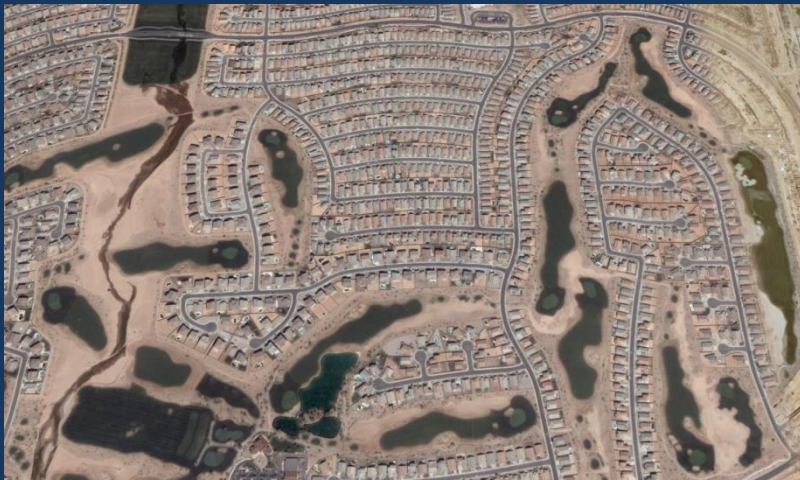
Increasing human populations are changing the Arizona landscape drastically; **top 10 fastest growing states**



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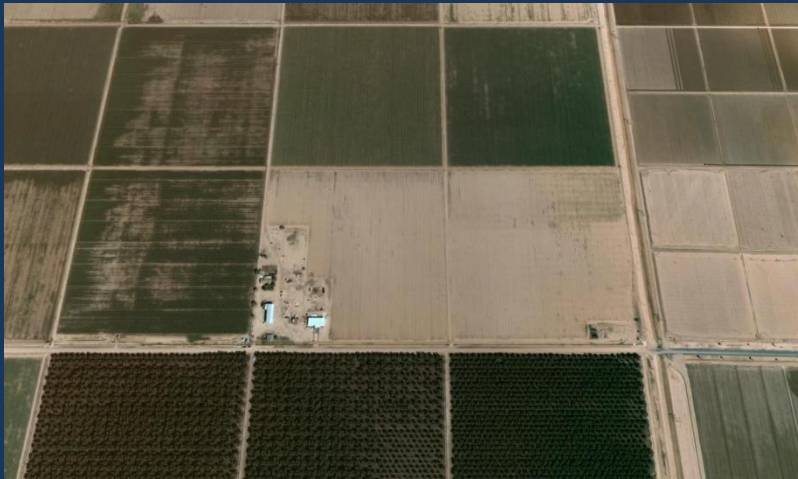


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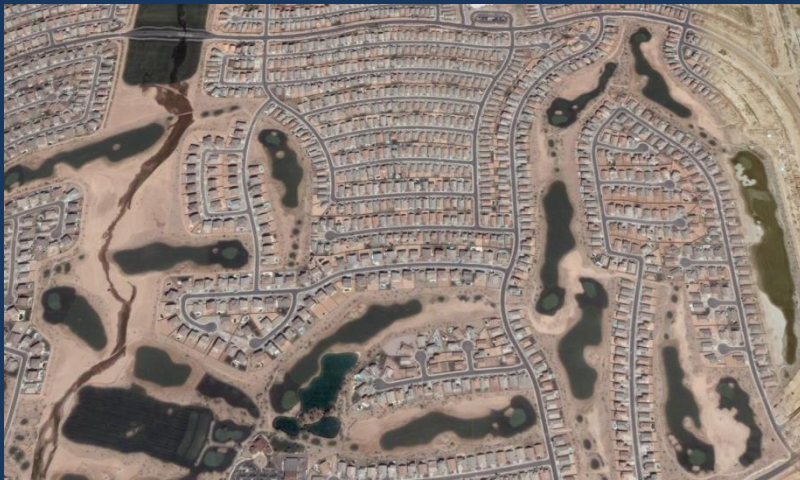


25% population increase
2000-2015 (2nd only to Nevada)

Why Water Recycling?



Increasing human populations are changing the Arizona landscape drastically; **top 10 fastest growing states**



25% population increase 2000-2015 (2nd only to Nevada)

Projected **60% additional increase** by 2050*

Why Water Recycling?

Arizona Annual Water Availability

Source	Availability (MAF)	Percent of Total
Surface Waters (Colorado, Gila, Salt Rivers)	4.0	50
Groundwater	3.5	43
Recycled Water	0.5	7

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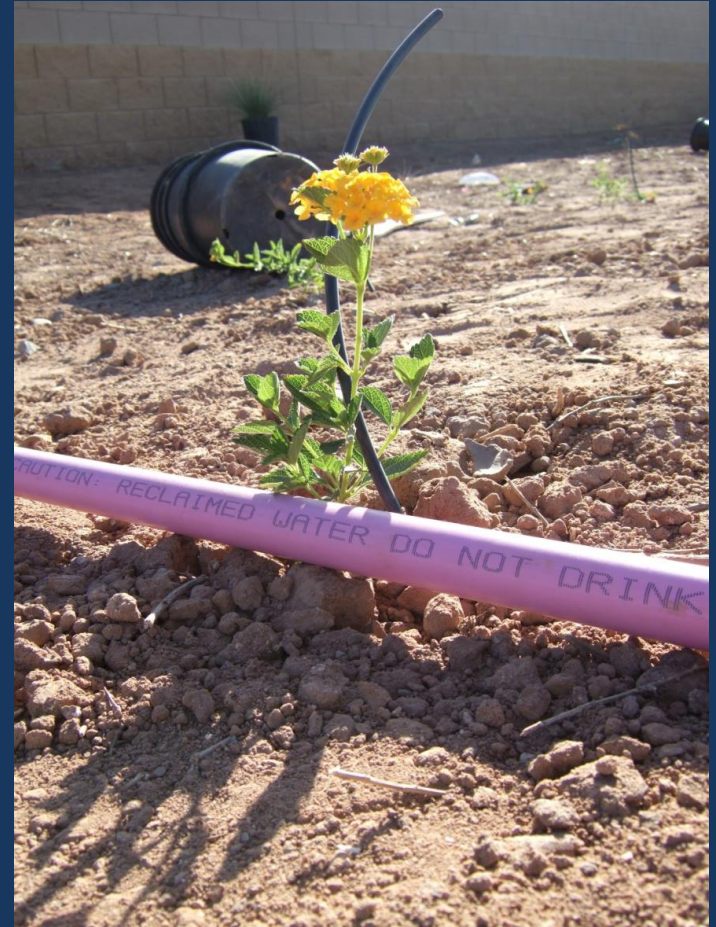
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Direct Potable Reuse: Should We Worry?

Biggest concerns:

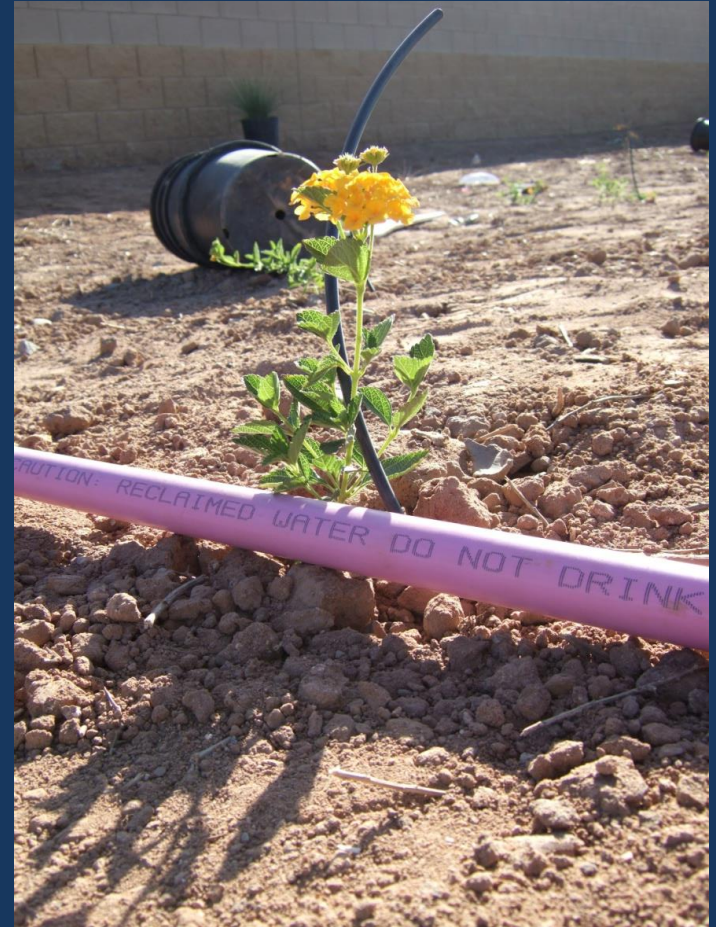
- Within-distribution system **microbial regrowth**
- Contaminants of concern



Direct Potable Reuse: Should We Worry?

Biggest concerns:

- Within-distribution system **microbial regrowth**
- Contaminants of concern
- **Chemical** (e.g., disinfection residual, PPCPs)
- **Biological** (e.g., resistance genes)



Chemicals of Concern

Thousands of constituents of concern, from pharmaceuticals (sulfamethoxazole), to insecticides (DEET), to personal care product ingredients (triclosan)

Ground water, surface waters, drinking water








Chemicals of Concern

(1) Pharmaceuticals & Personal Care Products - PPCP	(2) How Used/Where Found	(3) Acceptable (safe) vs. Actual Concentrations ug/l	(4) Relative Exposure at Actual Concentrations
Ibuprofen 	Over the counter (OTC) non-steroidal anti-inflammatory pain reliever (NSAID)	Acceptable = 890 Actual = 0.5	Our child could romp on the playground for 67,000 years before being exposed to the equivalent of one Advil tablet
17-beta estradiol 	Prescription hormone replacement	Acceptable = 0.39 Actual = 0.0084	After 160,000 years the child would be exposed to the equivalent of one dose of this hormone as it is typically prescribed
Fluoxetine 	Prescription antidepressant	Acceptable = 180 Actual = 0.031	After 220,000 years on the park lawn the child will have been exposed to the fluoxetine equivalent of one Prozac tablet
Sulfa- methoxazole 	Antibiotic commonly used to treat urinary tract infections or sinusitis	Acceptable = 70,000 Actual = 1.4	After 1,900,000 years at play, the child will have been exposed to the equivalent of one prescription dose of this antibiotic
PFOS 	Man-made fluorosurfactant formerly found in Scotchgard, numerous stain repellents, textiles, paper, and leather; in wax, polishes, paints, varnishes, and cleaning products for general use; in metal surfaces, and carpets	Acceptable = 630 Actual = 0.09	Our child can play for 46 years before he/she reaches the same exposure to PFOS it is estimated he/she receives in one day from other environmental factors
Bisphenol A 	Commonly called BPA; an organic compound known to be estrogenic; used to make polycarbonate plastic (water bottles) and epoxy resins, along with other applications	Acceptable = 1,300 Actual = 0.29	After 22 years at the park, the child will be exposed to the equivalent dose of BPA it is estimated he/she ingests from food in just one day
DEET 	N,N-diethyl-meta-toluamide (DEET) is the active ingredient in many insect repellent products	Acceptable = 18,000 Actual = 1.5	After playing on the wet lawn for 110 million years, the child will be exposed to the equivalent of one application of Deep Woods Sportsman Off to arms, hands and lower legs
Triclosan 	Antibacterial agent found in soap, toothpaste, deodorant; and is infused in an increasing number of con- sumer products, such as kitchen utensils, toys, bedding, socks, and trash bags	Acceptable = 1,400 Actual = 0.49	It would take 17,000 years on the playground before our child is exposed to the equivalent amount of Triclosan that he/she would get from washing his/her hands with anti-bacterial soap for 30 seconds
Acetaminophen 	OTC pain reliever	Acceptable = 57,000 Actual = 0.55	It would take 3,000,000 years of play before the child is exposed to the equivalent of one Extra-strength Tylenol tablet
Caffeine 	Stimulant found in coffee, tea, chocolate, and other food items	Acceptable = 30,000,000,000 Actual = 0.90	To be exposed to the same amount of caffeine found in a typical cup of coffee, our child will have to play for 410,000 years

*WaterReuse
Research
Foundation,
2011






Chemicals of Concern

Ibuprofen: 67,000 years to be exposed to a single dose

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17-β-Estradiol: 160,000 years

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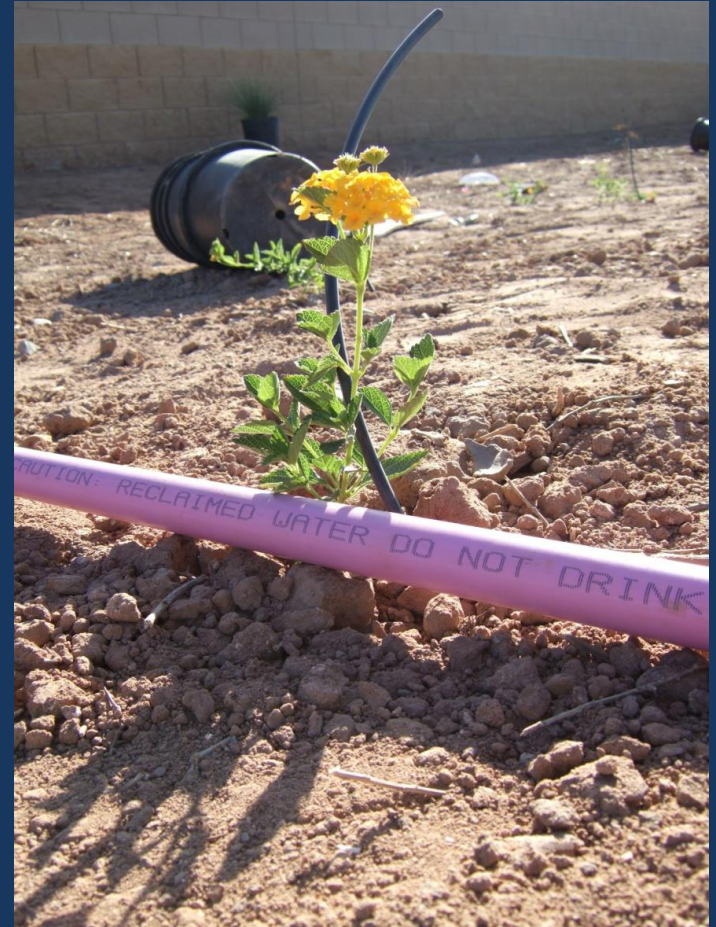
Chemicals of Concern

Sulfamethoxazole: 1,900,000 years

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Environmental and Public Health: Should We Worry?

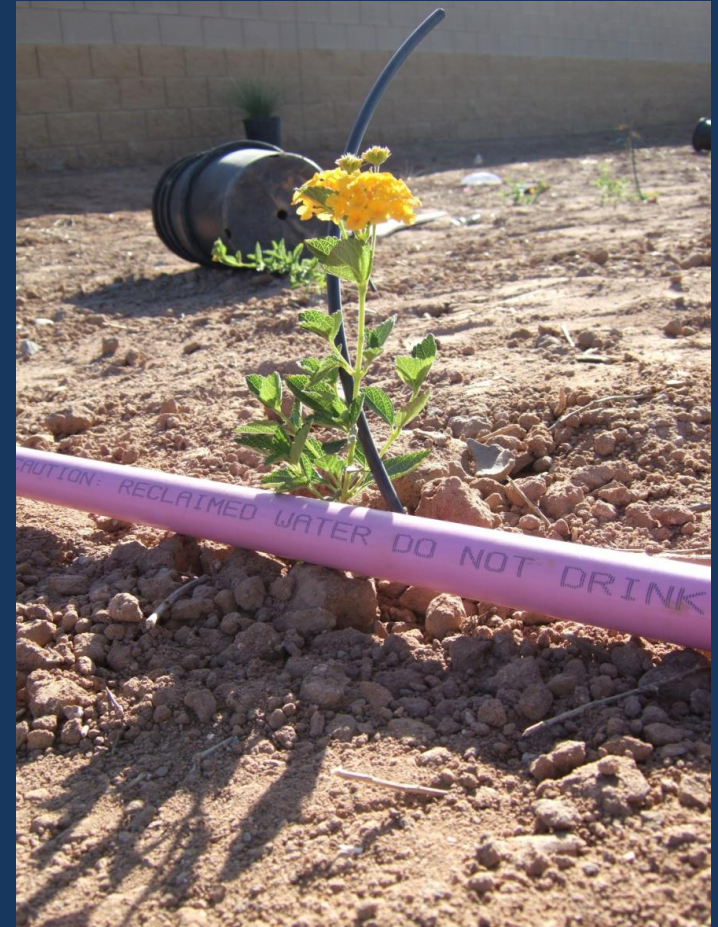
Acute effects of contaminants
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Chronic effects?

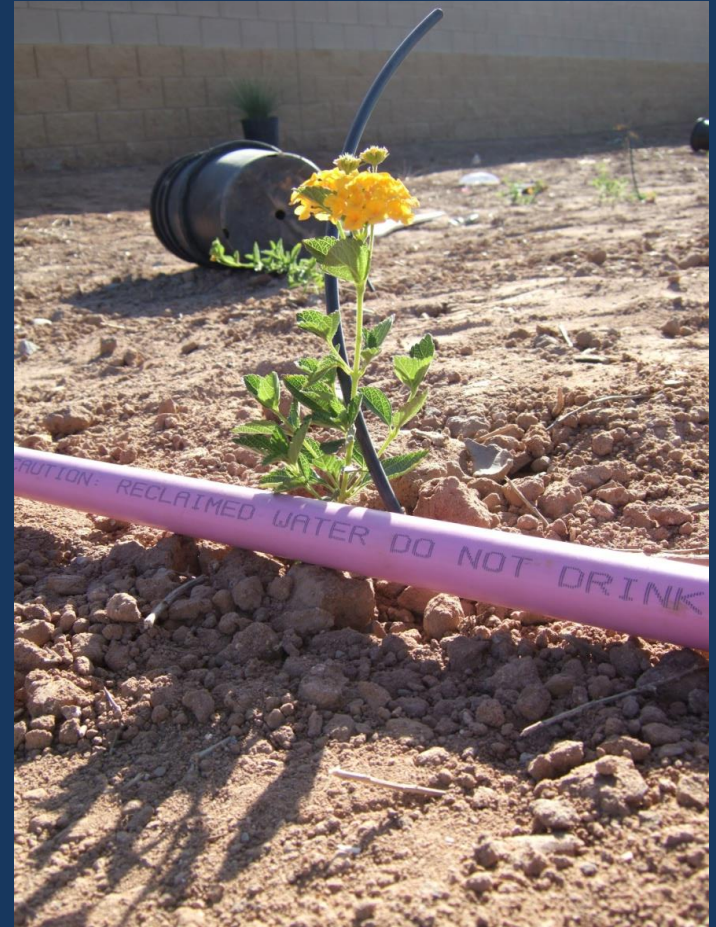


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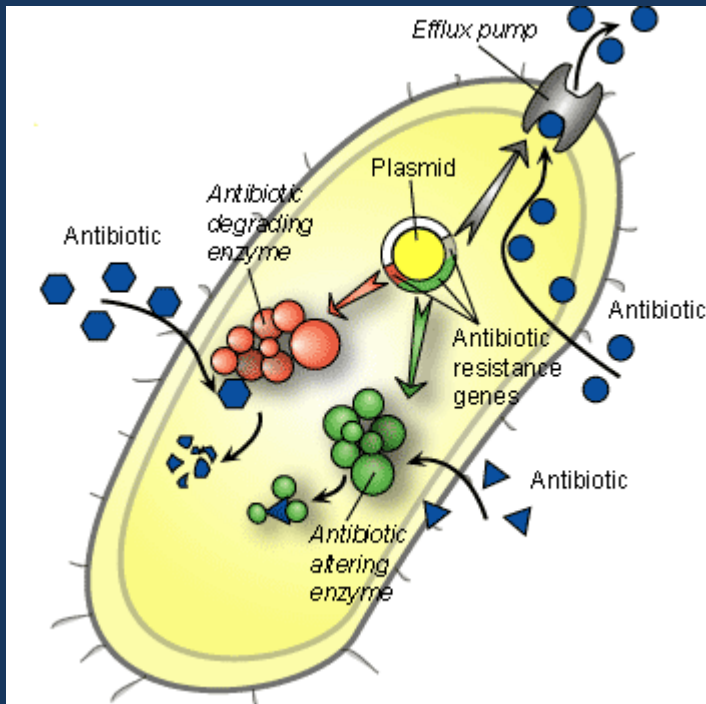
Chronic effects?

The case of antibiotic resistance



Antibiotic Resistant (ABR) Bacteria

The ability of a bacterium to **prevent an antibiotic from adversely affecting** that isolate, strain, or group.

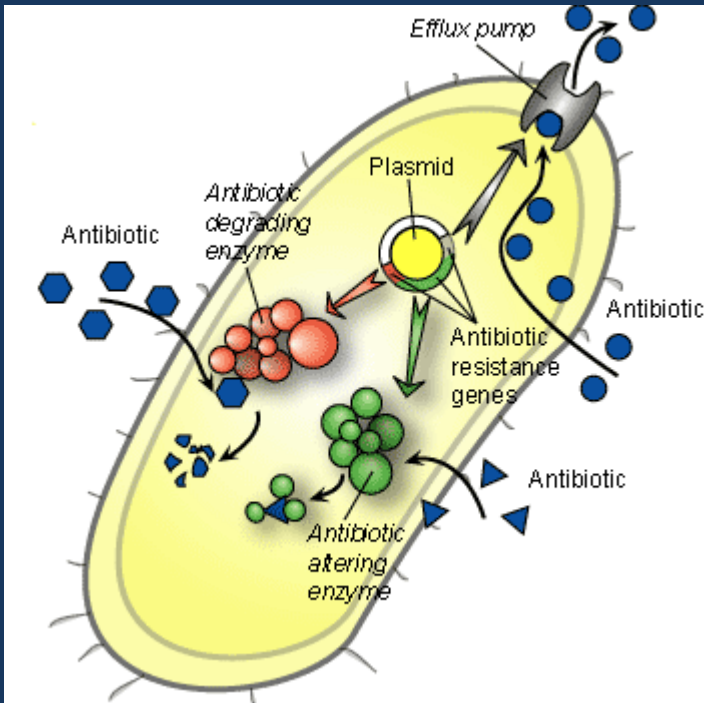


Antibiotic Resistant (ABR) Bacteria

The ability of a bacterium to **prevent an antibiotic from adversely affecting** that isolate, strain, or group.

Horizontal Gene Transfer confers antibiotic resistance in response to selective pressure

Clinical settings
High antibiotic dosages
Simplified microbial communities



Environmental Antibiotic Resistance

Agricultural and clinical use of antibiotics

Up to 75% excreted unaltered or as metabolites



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Many WWTPs not designed for the removal of these micropollutants

Recycled wastewater
Biosolids



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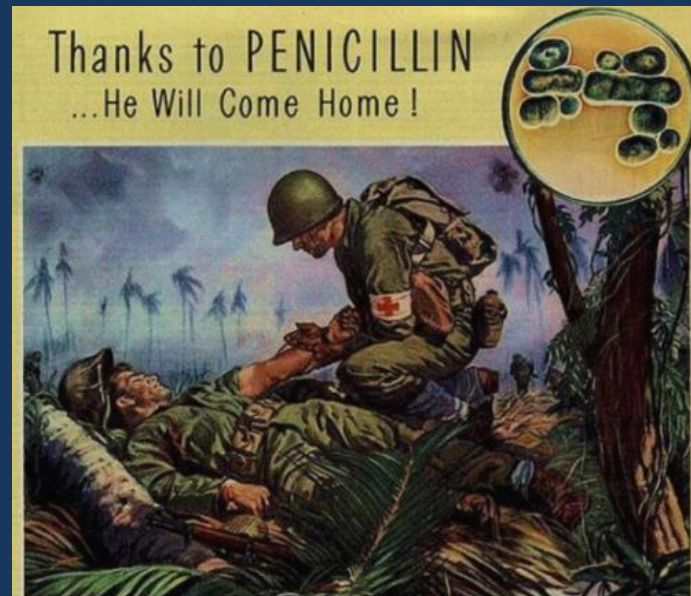
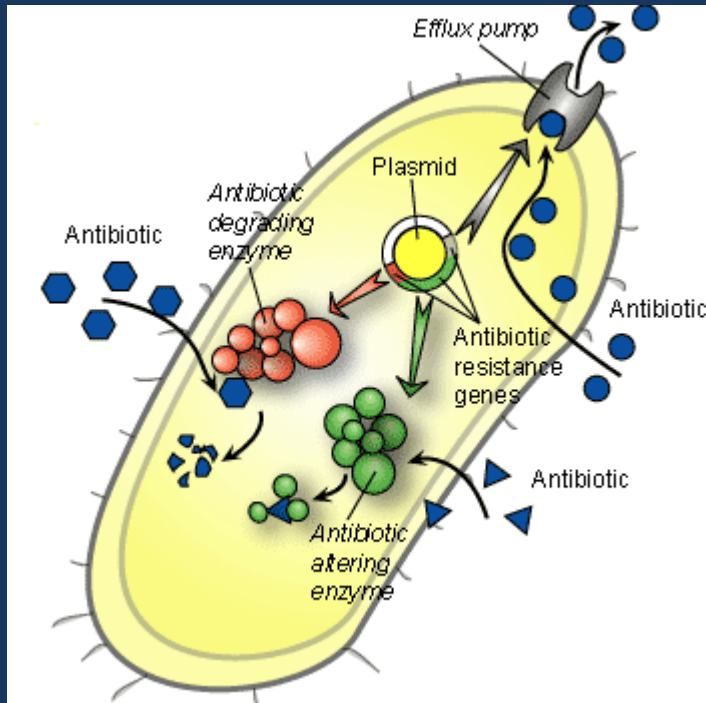
Recycled wastewater
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Proposed that this is a “key source of resistance to the environment”



Antibiotic Resistant (ABR) Bacteria

Earliest antibiotics: **naturally produced** (e.g., Penicillin)

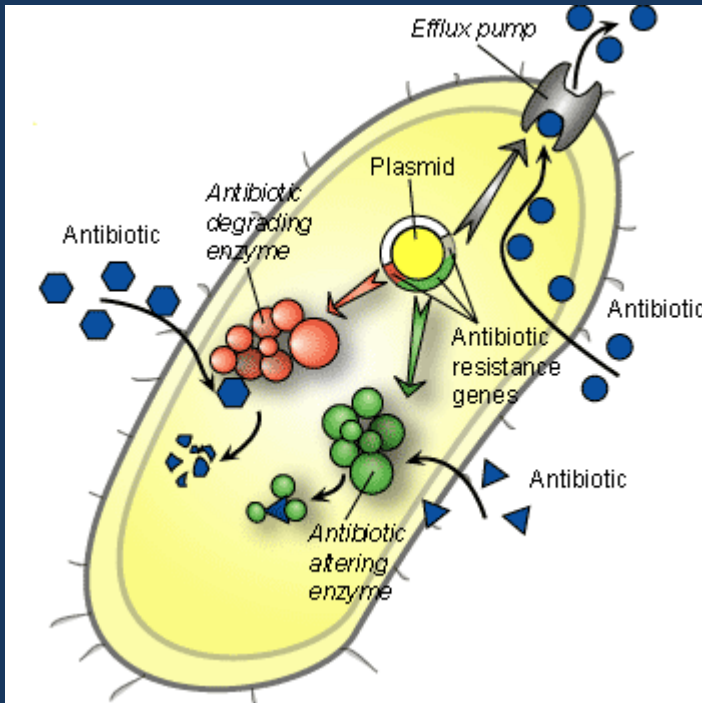


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ABR bacteria isolated from **deep terrestrial subsurface** (>250 m deep caves) and prehistoric (120,000+ YBP) **ice cores**

ABR genes evolved in **absence of selective pressure from humans**

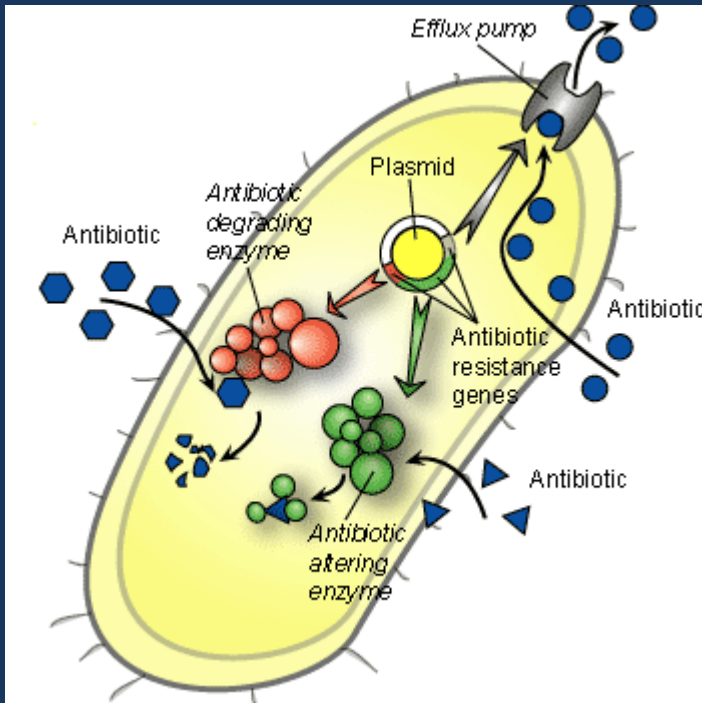


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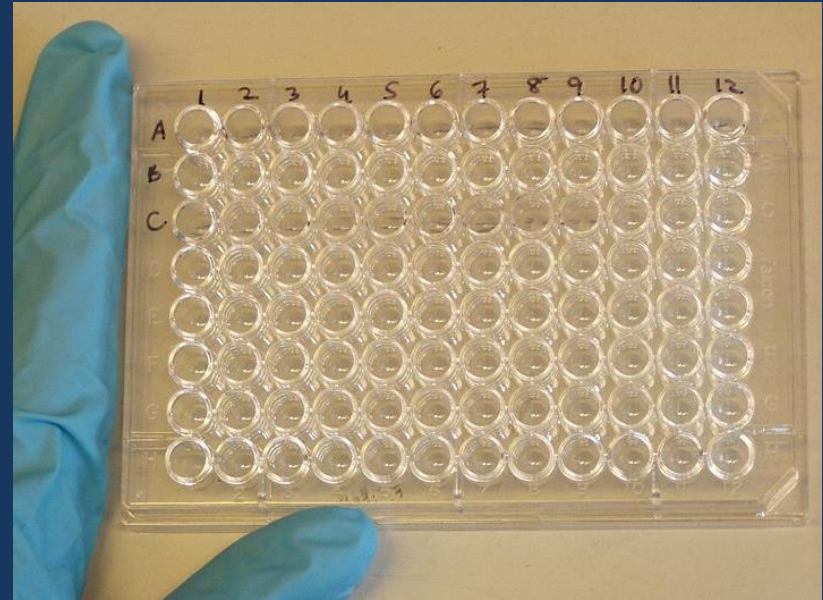
Detoxification
Competition for scarce
resources

Resistance Analysis Methods: Bacterial Isolation/Culturing

Broth microdilution methods

Agar disc diffusion

Clinical and Laboratory
Standards Institute



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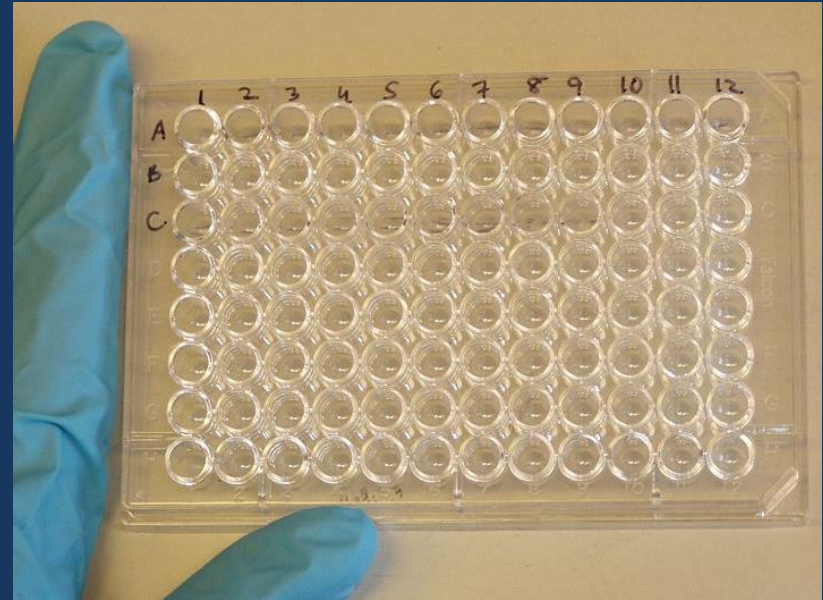
PROS:

Highly reproducible, robust

Can assess at clinical levels

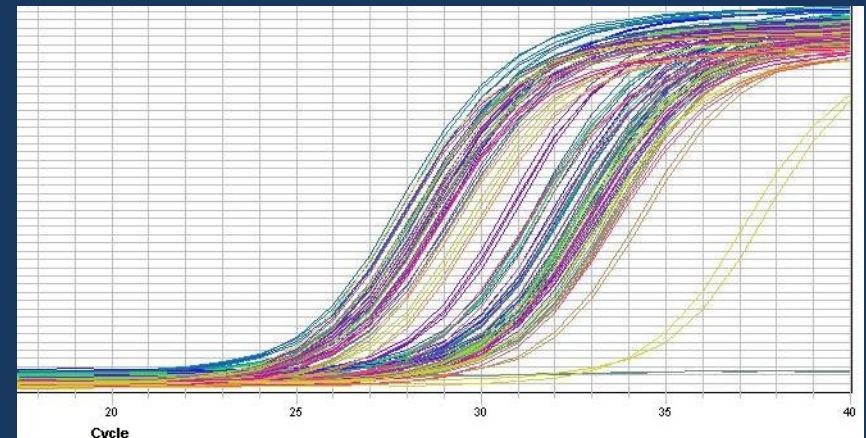
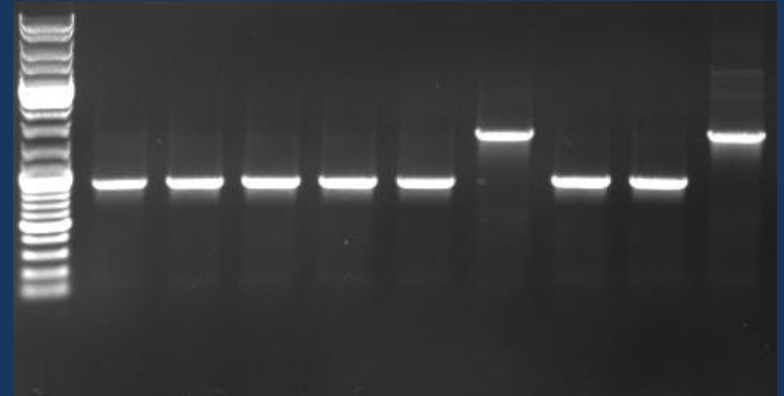
CONS:

Analyze a single isolate in each
plate



Resistance Analysis Methods: Molecular ID of Genes

Polymerase Chain Reaction (PCR), Quantitative PCR



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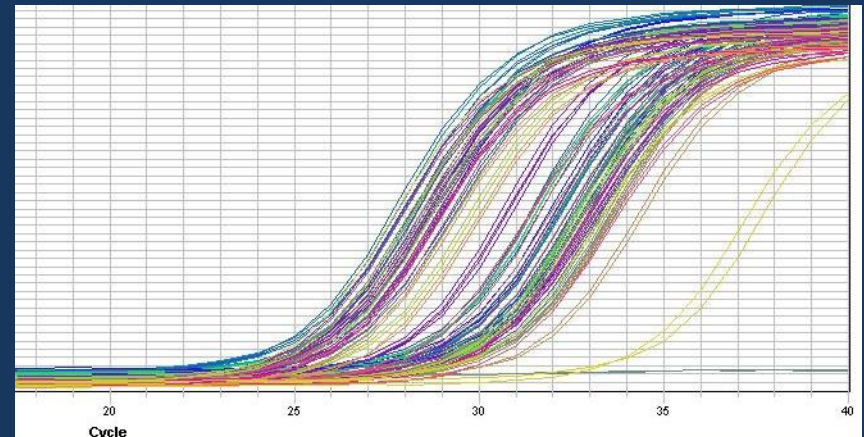
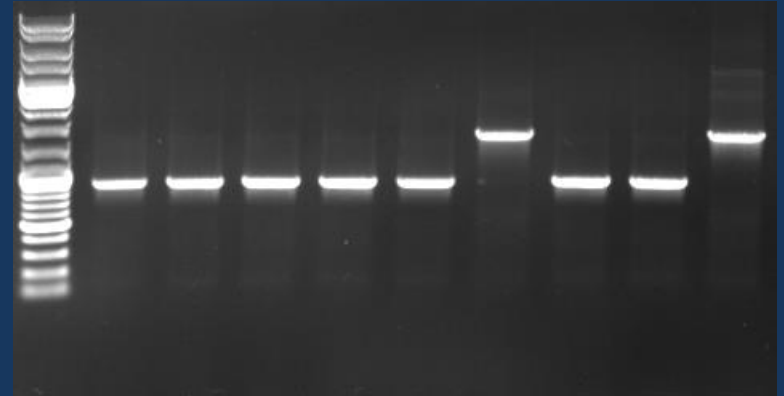
PROS:

Can identify/quantify resistance genes in entire DNA sample

Ability to quantify to single gene marker level

CONS:

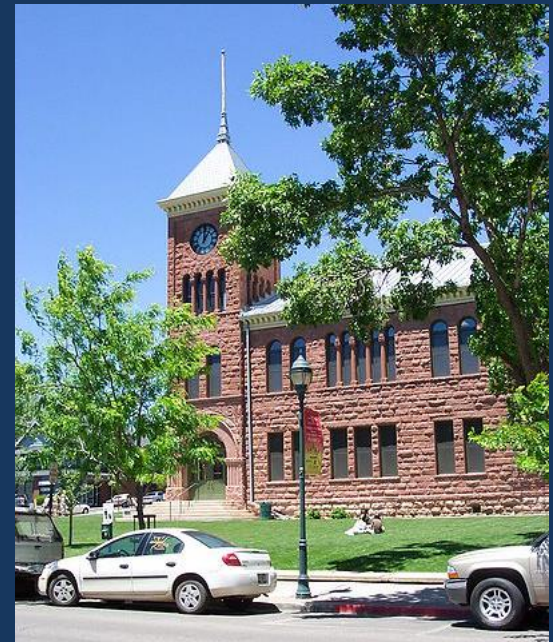
No discrimination between live and dead bacteria



How Did ABR Become Connected to Recycled Water Use?

The Case of Flagstaff, Arizona

- 4th largest metro area in Arizona; population ~68,000
- Elevation 6910 ft (2106 m)
- Magnet for outdoor enthusiasts



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- Major legal battle with Hopi tribes regarding use of recycled wastewater for snowmaking



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The Case of Flagstaff, Arizona

- 4th largest metro area in Arizona; population ~68,000
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- Magnet for outdoor enthusiasts
- Major legal battle with Hopi tribes regarding use of recycled wastewater for snowmaking
- Heightened public attention to a very sensitive issue



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Oct 17: “Antibiotic Resistance Racing Downriver”



More Recently (March 2016) The Case of Los Angeles and CRE

CRE thrives in soil and water AND in the
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Los Angeles Times

- “Deadly Superbugs Get Stronger in the Sewers...”
- WWTPs serve as a “luxury hotel” for superbugs
- “Chlorine is just not doing it”



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The Facts

- EPA sampled “partially treated wastewater”
- Antibiotic resistance genes



Antibiotic Resistance Genes and “Free DNA”

Water Environment Research
Foundation Study (2014-2017) by
Gerrity, Rock, and McLain

- Wastewater treatment under
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- Wastewater treatment under a range of SRTs
- As much as 50% of total DNA was “free DNA”
- Quantified intact resistance genes by PCR



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Flagstaff City Manager's Expert Panel
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2. Recycled water pipe only: what about drinking/potable water pipes?



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Recycled water is an easy target –
well-controlled research is essential
to informed decision making



Does Recycled Municipal Wastewater Induce Antibiotic Resistance?



Gilbert Riparian Preserve

Created in 1986, seven
recharge basins receive
tertiary-treated recycled water



Gilbert Riparian Preserve

Created in 1986, seven
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Control site: agricultural
irrigation retention pond



Field Sampling over Two Years

Soil cores removed
from sediments of
retention basins

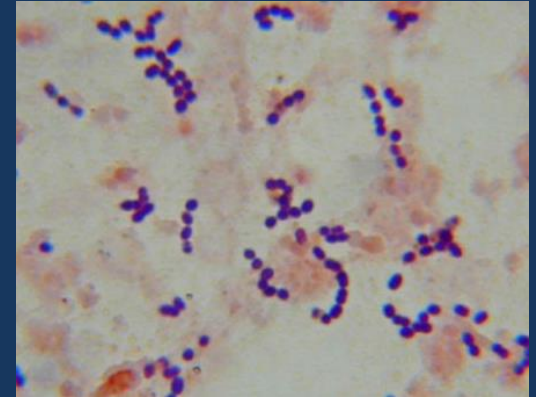
Selective enrichment
for **Gram-positive
enterococci**



Field Sampling over Two Years

Enterococcus spp.

GI tracts of humans and animals;
environmental persistence



Field Sampling over Two Years

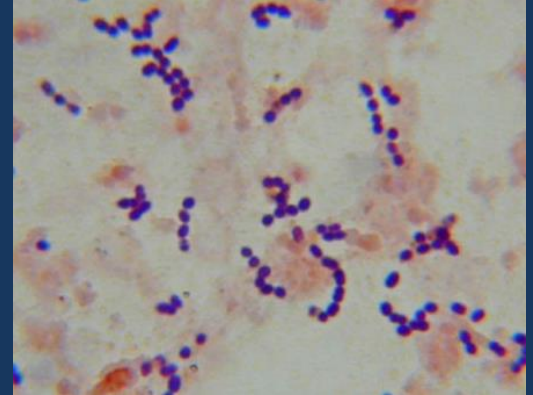
Enterococcus spp.

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Great capacity for **gene transfer**

Emergence of multiple drug-
resistant strains in clinical settings

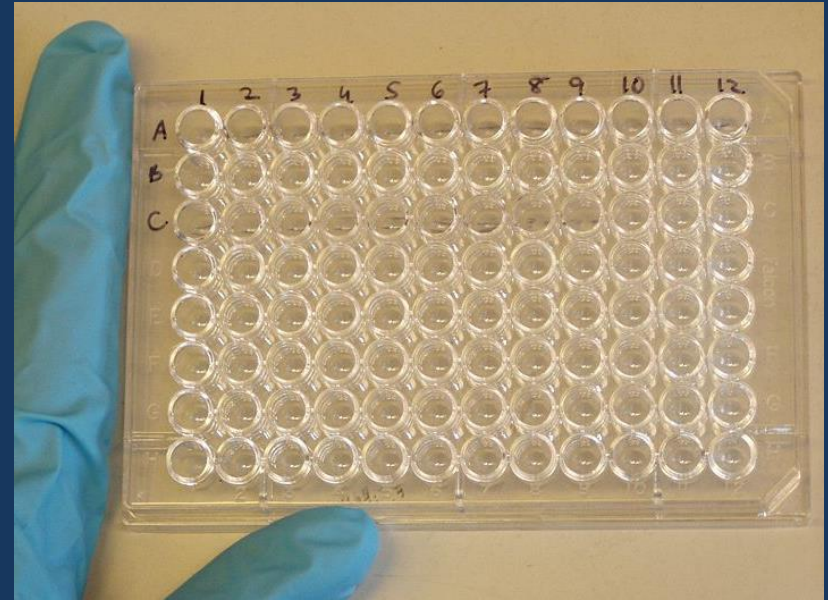
Ideal bacterial group for
investigating the ecology of AR
development



Laboratory Methods

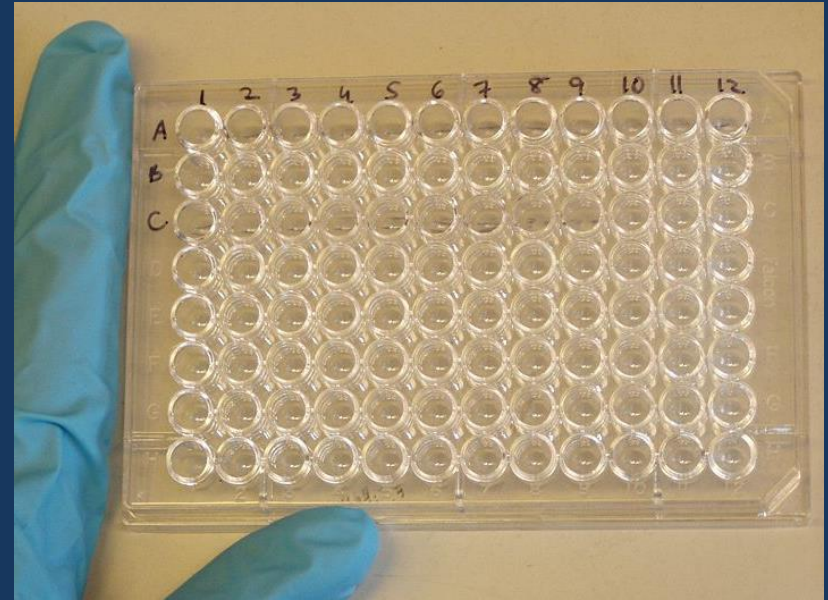
Isolates tested for resistance to 16 widely used antibiotics using **CLSI broth microdilution methods**

	1	2	3	4	5	6	7	8	9	10	11	12
A	TGC 0.005	TGC 0.008	TGC 0.006	TGC 0.012	TGC 0.025	TGC 0.5	ERY 0.25	ERY 0.5	ERY 1	ERY 2	ERY 4	ERY 8
B	TET 1	TET 2	TET 4	TET 8	TET 16	TET 32	CIP 0.12	CIP 0.25	CIP 0.5	CIP 1	CIP 2	CIP 4
C	CHL 2	CHL 4	CHL 8	CHL 16	CHL 32	PEN 0.25	PRN 0.5	PRN 1	PRN 2	PEN 4	PRN 8	PRN 16
D	DAP 0.25	DAP 0.5	DAP 1	DAP 2	DAP 4	DAP 8	DAP 16	VAN 0.25	VAN 0.5	VAN 1	VAN 2	VAN 4
E	STR 512	STR 1024	STR 2048	MIT 2	MIT 4	MIT 8	MIT 16	MIT 32	MIT 64	VAN 8	VAN 16	VAN 32
F	TYLT 0.25	TYLT 0.5	TYLT 1	TYLT 2	TYLT 4	TYLT 8	TYLT 16	TYLT 32	GEN 128	GEN 256	GEN 512	GEN 1024
G	SIN 0.5	SIN 1	SIN 2	SIN 4	SIN 8	SIN 16	SIN 32	LIN 1	LIN 2	LIN 4	LIN 8	NEG
H	LED 0.5	LED 1	LED 2	LED 4	LED 8	KAN 128	KAN 256	KAN 512	KAN 1024	POS	POS	POS



Laboratory Methods

Isolates tested for resistance to 16 widely used antibiotics using **CLSI broth microdilution methods**



	1	2	3	4	5	6	7	8	9	10	11	12
A	TGC	TGC	TGC	TGC	TGC	TGC	ERY	ERY	ERY	ERY	ERY	ERY
B	TET	TET	TET	TET	TET	TET	CIP	CIP	CIP	CIP	CIP	CIP
C	CHL	CHL	CHL	CHL	CHL	PEN	PEN	PEN	PEN	PEN	PEN	PEN
D	DAP	DAP	DAP	DAP	DAP	DAP	VAN	VAN	VAN	VAN	VAN	VAN
E	STR	STR	STR	MIT	MIT	MIT	MIT	MIT	MIT	VAN	VAN	VAN
F	TYLT	TYLT	TYLT	TYLT	TYLT	TYLT	TYLT	TYLT	GEN	GEN	GEN	GEN
G	SMN	SMN	SMN	SMN	SMN	SMN	SMN	LMN	LMN	LMN	LMN	NEG
H	LED	LED	LED	LED	LED	RMN	RMN	RMN	RMN	POS	POS	POS

	0.015	0.03	0.06	0.12	0.25	0.5	1
B	TET	TET	TET	TET	TET	TET	TET
	1	2	4	8	16	32	64

High Level Antibiotic Resistance

Antimicrobial	% Isolates with High-Level Resistance	
	Groundwater	Wastewater
Tigecycline	14.3	6.1
Erythromycin	42.8	21.2
Tetracycline	21.4	0.0
Ciprofloxacin	57.1	24.2
Chloramphenicol	7.1	0.0
Penicillin	14.3	6.1
Daptomycin	57.1	51.5
Vancomycin	7.1	3.0
Streptomycin	0.0	0.0
Nitrofurantoin	28.6	21.2
Tylosin Tartrate	25.0	3.0
Gentamycin	0.0	0.0
Quinupristin/Dalfopristin	3.6	27.3
Lincomycin	57.1	48.5
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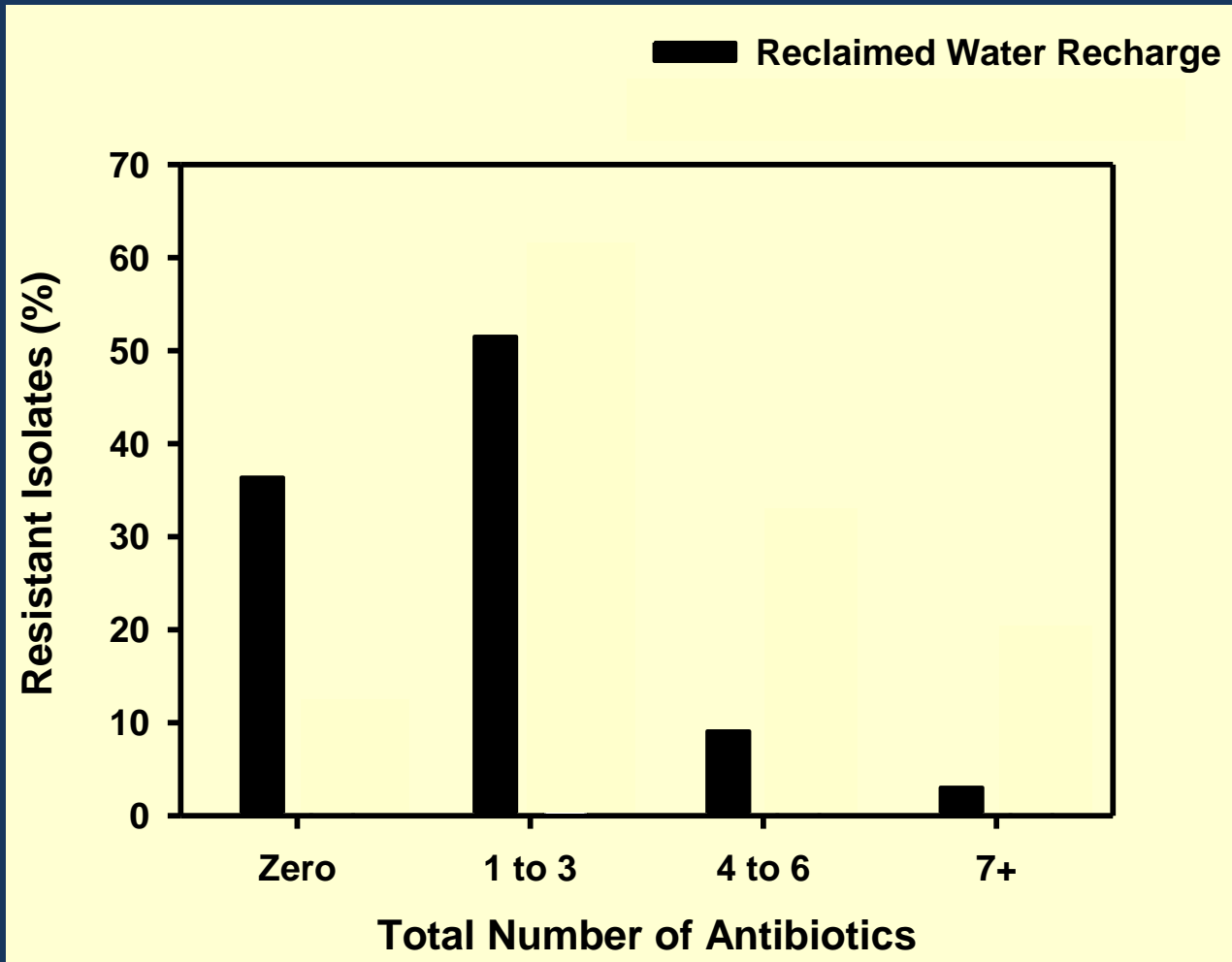
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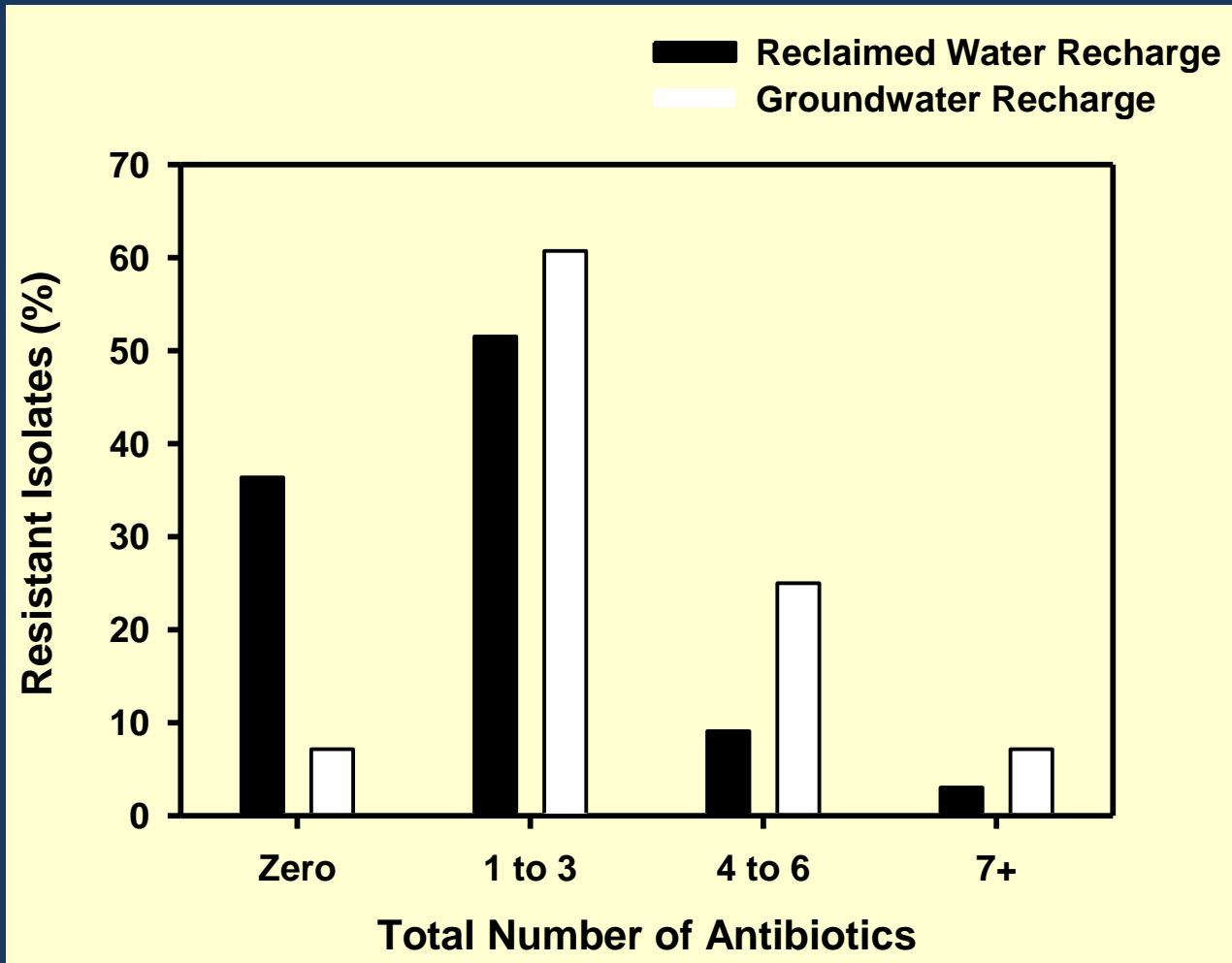
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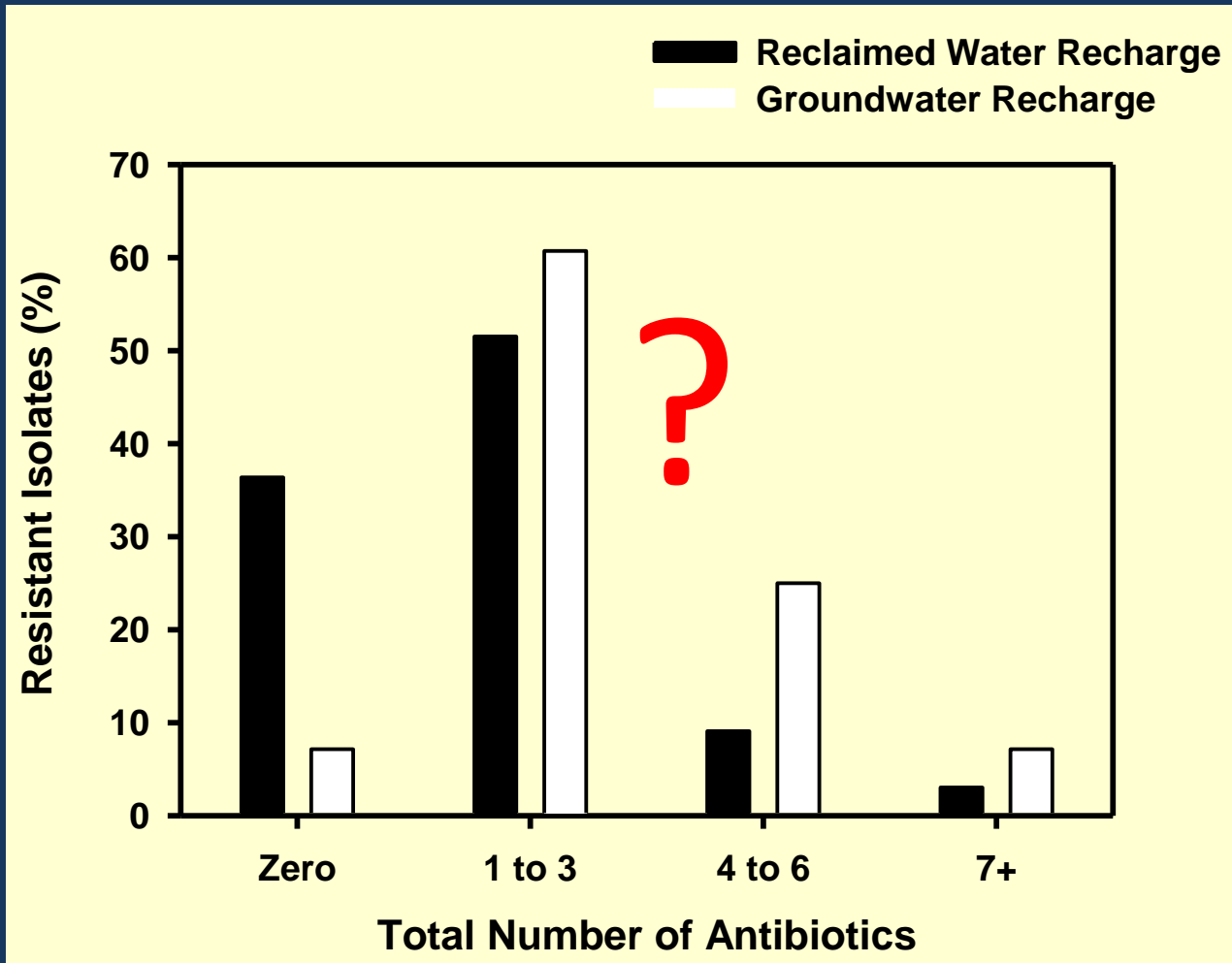
Multi-Antibiotic Resistance – “Superbugs”



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Multi-Antibiotic Resistance- “Superbugs”



What Does This Mean?

Antibiotic resistance:
“metabolically expensive”



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Recycled Water, Biosolids = Adding
Soil Carbon

Does adding C at certain levels
reduce bacterial competition and
overcome the need to express
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“metabolically expensive”

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Is this unique to semi-arid soils?



Does Recycled Municipal Wastewater Induce Antibiotic Resistance in Environmental Bacteria?



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Unknown.



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Published reports indicate that recycled wastewater and feedlot runoff may **select for resistance in environmental bacteria**



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50 studies published in 2013:
29 lacked a true control site



Antibiotic Resistance in Treated Wastewater

World Health Organization: “one of the most critical human health challenges of the next century



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Press Reports:

How was this data derived?

Remember – **treated wastewater** is an easy target



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mclainj@email.arizona.edu

